

**WHAT IS CLAIMED IS:**

1. An isolated enone reductase having the physicochemical properties of (A)-(C):
  - (A) it reduces the carbon-carbon double bond of an  $\alpha,\beta$ -unsaturated ketone, using NADPH as an electron donor, to produce a corresponding saturated hydrocarbon;
  - (B) it has a substrate specificity of (1)-(4):
    - (1) it has substantially no activity to reduce the keto group of a ketone;
    - (2) it exhibits a significantly higher activity with NADPH than with NADH as an electron donor;
    - (3) it does not substantially act on substrates wherein both substituents at the  $\beta$  carbon relative to the ketone are not hydrogen; and
    - (4) it does not substantially act on a substrate in which the carbon-carbon double bond is present in a cyclic structure; and
  - (C) it has an optimal pH of 6.5-7.0.
2. The enone reductase of claim 1, wherein the reductase (a) has an optimum temperature of 37-45°C; and (b) has a molecular weight determined by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and by gel filtration of about 43,000 and about 42,000, respectively.
3. The enone reductase of claim 1, which is derived from an organism of the genus *Kluyveromyces*.
4. A method for obtaining an enone reductase, comprising the step of (a) culturing a microorganism belonging to the genus *Kluyveromyces*; and (b) isolating the enone reductase of claim 1 from the cultured microorganism.
5. The method of claim 4, wherein the microorganism belonging to the genus *Kluyveromyces* is *Kluyveromyces lactis*.
6. An isolated nucleic acid of any one of (a) to (d) below:
  - (a) a nucleic acid encoding a protein comprising the amino acid sequence of SEQ ID NO:2;

4 (b) a nucleic acid comprising a coding region of the nucleotide sequence of  
5 SEQ ID NO:1;

6 (c) a nucleic acid encoding a protein that comprises the amino acid sequence  
7 of SEQ ID NO: 2, in which one or more amino acids are substituted, deleted, inserted and/or  
8 added and that is functionally equivalent to a protein consisting of the amino acid sequence  
9 of SEQ ID NO: 2;

10 (d) a nucleic acid that hybridizes under stringent conditions with a nucleic acid  
11 consisting of the nucleotide sequence of SEQ ID NO: 1, and that encodes a protein  
12 functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO:2;  
13 and

14 (e) a nucleic acid encoding a protein that has at least 60% identity to the amino acid  
15 sequence of SEQ ID NO:2.

1 7. An isolated nucleic acid encoding the amino acid sequence of SEQ ID NO:2  
2 or a fragment thereof.

1 8. A vector comprising the nucleic acid of claim 6.

1 9. A vector comprising the nucleic acid of claim 7.

1 10. The vector of claim 8, further comprising a nucleic acid sequence encoding a  
2 dehydrogenase that catalyzes oxidation-reduction reactions using NADP as a coenzyme.

1 11. The vector of claim 9, further comprising a nucleic acid sequence encoding a  
2 dehydrogenase that catalyzes oxidation-reduction reactions using NADP as a coenzyme.

1 12. A transformant harboring the nucleic acid of claim 6.

1 13. A transformant harboring the nucleic acid of claim 7.

1 14. A transformant harboring the vector of claim 8.

1 15. A transformant harboring the vector of claim 10.

1 16. A substantially purified polypeptide encoded by the nucleic acid of claim 6.

1 17. A substantially purified polypeptide encoded by the nucleic acid of claim 7.

1 18. A method for producing a polypeptide, the method comprising the steps of  
2 culturing the transformant of claim 12 and recovering a polypeptide expressed from the  
3 transformant or the culture supernatant thereof.

1 19. A method for producing a polypeptide, the method comprising the steps of  
2 culturing the transformant of claim 13 and recovering a polypeptide expressed from the  
3 transformant or the culture supernatant thereof.

1 20. A method for producing a polypeptide, the method comprising the steps of  
2 culturing the transformant of claim 14 and recovering a polypeptide expressed from the  
3 transformant or the culture supernatant thereof.

1 21. A method for producing a polypeptide, the method comprising the steps of  
2 culturing the transformant of claim 15 and recovering a polypeptide expressed from the  
3 transformant or the culture supernatant thereof.

1 22. An isolated nucleic acid of any one of (a) to (d) below:

2 (a) a nucleic acid encoding a protein comprising the amino acid sequence of  
3 SEQ ID NO:4, 6 or 8;

4 (b) a nucleic acid comprising a coding region of the nucleotide sequence of  
5 SEQ ID NO:3, 5 or 7;

6 (c) a nucleic acid encoding a protein that comprises the amino acid sequence  
7 of SEQ ID NO:4, 6 or 8 in which one or more amino acids are substituted, deleted, inserted  
8 and/or added and that is functionally equivalent to a protein consisting of the amino acid  
9 sequence of SEQ ID NO:4, 6 or 8;

10 (d) a nucleic acid that hybridizes under stringent conditions with the nucleic acid  
11 consisting of the nucleotide sequence of SEQ ID NO: 3, 5 or 7, and that encodes a protein  
12 functionally equivalent to a protein consisting of the amino acid sequence of SEQ ID NO:4, 6  
13 or 8; and

14 (e) a nucleic acid encoding a protein that has at least 60% identity to the amino acid  
15 sequence of SEQ ID NO:4, 6 or 8.

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- 1 23. A substantially purified polypeptide encoded by the nucleic acid of claim 22.
- 1 24. A vector comprising the nucleic acid of claim 22.
- 1 25. The vector of claim 24, further comprising a nucleic acid sequence encoding a  
2 dehydrogenase that catalyzes oxidation-reduction reactions using NADP as a coenzyme.
- 1 26. A transformant harboring the nucleic acid of claim 2.
- 1 27. A transformant harboring the vector of claim 24.
- 1 28. A transformant harboring the vector of claim 25.
- 1 29. A method for producing a polypeptide, the method comprising the steps of  
2 culturing the transformant of claim 26 and recovering a polypeptide expressed from the  
3 transformant or the culture supernatant thereof.
- 1 30. A method for producing a polypeptide, the method comprising the steps of  
2 culturing the transformant of claim 27 and recovering a polypeptide expressed from the  
3 transformant or the culture supernatant thereof.
- 1 31. A method for selectively reducing the carbon-carbon double bond of an  
2  $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with the  
3 enone reductase of claim 1.
- 1 32. A method for selectively reducing the carbon-carbon double bond of an  
2  $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with the  
3 polypeptide of claim 16.
- 1 33. A method for selectively reducing the carbon-carbon double bond of an  
2  $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with the  
3 polypeptide of claim 17.
- 1 34. A method for selectively reducing the carbon-carbon double bond of an  
2  $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with the  
3 polypeptide of claim 23.

35. A method for selectively reducing the carbon-carbon double bond of an  $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with a microorganism that produces an enone reductase having the physicochemical properties of (A)-(C):

(A) it reduces the carbon-carbon double bond of an  $\alpha,\beta$ -unsaturated ketone, using NADPH as an electron donor, to produce a corresponding saturated hydrocarbon;

(B) it has a substrate specificity of (1)-(4):

(1) it has substantially no activity to reduce the keto group of a ketone;

(2) it exhibits a significantly higher activity with NADPH than with

NADH as an electron donor;

(3) it does not substantially act on substrates wherein both substituents at the  $\beta$  carbon relative to the ketone are not hydrogen; and

(4) it does not substantially act on a substrate in which the carbon-carbon double bond is present in a cyclic structure; and

(C) it has an optimal pH of 6.5-7.0.

36. The method of claim 35, wherein the microorganism is of the genus

*Kluyveromyces*.

37. The method of claim 35, wherein the microorganism is the transformant of claim 12.

38. The method of claim 35, wherein the microorganism is the transformant of claim 26.

39. A method for selectively reducing the carbon-carbon double bond of an  $\alpha,\beta$ -unsaturated ketone, comprising the step of reacting an  $\alpha,\beta$ -unsaturated ketone with a processed product of a microorganism that produces an enone reductase having the physicochemical properties of (A)-(C):

(A) it reduces the carbon-carbon double bond of an  $\alpha,\beta$ -unsaturated ketone, using NADPH as an electron donor, to produce a corresponding saturated hydrocarbon;

(B) it has a substrate specificity of (1)-(4):

(1) it has substantially no activity to reduce the keto group of a ketone;

9 (2) it exhibits a significantly higher activity with NADPH than with  
10 NADH as an electron donor;

11 (3) it does not substantially act on substrates wherein both substituents at  
12 the  $\beta$  carbon relative to the ketone are not hydrogen; and

13 (4) it does not substantially act on a substrate in which the carbon-carbon  
14 double bond is present in a cyclic structure; and

15 (C) it has an optimal pH of 6.5-7.0.

1 40. The method of claim 38, wherein the microorganism is of the genus  
2 *Kluyveromyces*.

1 41. The method of claim 38, wherein the microorganism is the transformant of  
2 claim 12.

1 42. The method of claim 38, wherein the microorganism is the transformant of  
2 claim 26.